Proteomics-based analysis of lung injury–induced proteins in a mouse model of common bile duct ligation

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Background. *Lung injury is a life-threatening complication in patients with liver dysfunction. We recently provided an experimental lung injury model in mouse with common bile duct ligation. In this study, we aimed to characterize the pathologic and biochemical features of lung tissues in common bile duct ligation mice using a proteomic approach.*

Methods. *Common bile ducts of BALB/c mice, 8 weeks of age, were ligated operatively. CD31-expressing pulmonary cells were sorted with immunomagnetic microbeads, and protein profiles were examined by 2-dimensional gel electrophoresis. Based on the results of protein identification, immunohistochemistry and quantitative reverse transcription polymerase chain reaction were carried out in pulmonary and hepatic tissues.*

Results. *Two-dimensional gel electrophoresis revealed 3 major inflammation-associated proteins exhibiting considerable increases in the number of CD31-positive pulmonary cells after common bile duct ligation. Mass spectrometry analysis identified these proteins as SerpinB1a (48 kDa), ANXA1 (46 kDa), and S100A9 (16 kDa). Furthermore, the 3 proteins were more highly expressed in dilated pulmonary blood vessels of common bile duct ligation mice, in which neutrophils and monocytes were prominent, as shown by immunohistochemistry. More importantly, SerpinB1a mRNA and protein were significantly upregulated in the liver, whereas S100A9 and ANXA1 mRNA and protein were upregulated in the lungs, as shown by quantitative reverse transcription polymerase chain reaction and Western blotting.*

Conclusion. *We identified 3 proteins that were highly expressed in the lung after common bile duct ligation using a proteomics-based approach. (Surgery 2016;■:■-■.)*

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Liver dysfunction, such as cirrhosis, induces vascular abnormalities in several organs, resulting in impairment of organ function and subsequent increases in morbidity and mortality.[1,2] Clinical studies have provided evidence of lung injury in patients with acute liver failure.[3-5] In such cases, severe hypoxemia due to increased pulmonary capillary permeability is often observed. Moreover, similar lung injury pathology has been investigated experimentally in a porcine model of acute liver failure.[6] These studies have shown that lung injury after liver injury can cause fatal complications, such as severe desaturation in blood and

intrapulmonary bleeding.[1] Therefore, there is an urgent need to establish effective medical approaches for managing lung damage.

In a recent study, we established an experimental murine lung injury model induced by common bile duct ligation (CBDL) and observed dilated vessels and angiogenesis in lungs after operation.[7] Immunohistologic analyses and gene expression profiling in pulmonary cells revealed a significant increase in CD31-positive vascular endothelial cells 2–3 weeks after CBDL, with modulation of 19 genes encoding angiogenesis-regulating factors. Notably, the lung pathology and gene expression patterns in our murine model showed significant differences compared with those in rat models, with regard to DNA microarray data. For example, in our mouse model, we found that tumor necrosis factor (TNF)-α signaling was strongly activated in proliferating CD31-positive pulmonary cells and played an important role in the development of pulmonary injuries and consequent vascular endothelial growth factor–independent angiogenesis.[7]

In this study, we investigated protein profiles in the lungs of CBDL mice, using a proteomic approach to obtain further insights into the molecular mechanisms underlying lung injury in patients with acute liver failure. Our findings may contribute to the development of medical treatments for lung injuries associated with cholestatic liver injury.

MATERIALS AND METHODS

Mouse model of CBDL. All mice were operated on and cared for following the animal protocols approved by the Ehime University Animal Care Committee (approval number: 05R02-2). Fifty-five Balb/c mice (8 weeks old) were purchased from Clea Japan Inc (Tokyo, Japan). Mice were provided a sterilized diet and water ad libitum. Common bile duct ligation and sample collection were performed as previously described.[7] Briefly, the common bile duct was dissected, doubly ligated with commercially available surgical sutures (7-0 Prolene sutures; Ethicon, Somerville, NJ), and divided into groups.

In control mice, dissection around the common bile duct was performed. All surviving mice were died between 2 and 4 weeks after operation. Before death, mice were anesthetized using a combination of ketamine (0.1 mg/g) and xylazine (0.01 mg/g) in phosphate-buffered saline (PBS). Under complete sedation, mice were subcutaneously injected with 50 IU heparin sodium. The trachea was cannulated using a 20-G Terumo Surflo Catheter (Terumo Corporation, Japan) to inflate the lungs. Pulmonary arteries were irrigated with 10 mL cold PBS and drained via the operatively opened right atrial appendage. After the inflation, the lungs were fixed at 23 cm pressure, using 4% paraformaldehyde. The other organs (hearts, livers, and spleens) were removed carefully for subsequent experiments.

Isolation of CD31-positive cells using magnetic beads. CD31-positive pulmonary cells were purified as described previously.[7] Briefly, pulmonary tissues were obtained and digested with 400 U/mL collagenase type I (Worthington, Lakewood, NJ). Antimouse CD31 antibody (BD Biosciences, San Diego, CA) was conjugated with Dynabeads Sheep antirat IgG (Life Technologies, Grand Island, NY). The digested tissues were passed through a 70-μm mesh filter and gently centrifuged. The pellet was suspended with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Wako Pure Chemical Industries, Osaka, Japan). The cells were mixed with anti-CD31 antibody-coated magnetic beads and then incubated for 20 min at 4°C. CD31-positive pulmonary cells were washed with DMEM.

Two-dimensional (2D) gel electrophoresis. 2D gel electrophoresis was performed as previously described (Fig 1, *A*).[8] Briefly, the purified CD31-positive pulmonary cells were lysed with urea lysis buffer containing 8.5 M urea, 4% CHAPS, and 0.2% Biolyte 3/10 (Bio-Rad Laboratories, Hercules, CA) for 2D gel electrophoresis. Cell lysates containing 20 μg total proteins were separated using Immobiline Dry Strips (13 cm in length, pH 3–10; GE Healthcare, Buckinghamshire, UK). Subsequently, as a second dimension, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 10% acrylamide gels. Proteins were visualized with SyproRuby (Invitrogen, Carlsbad, CA).

In-gel digestion and mass spectrometry (MS). In-gel tryptic digestion was performed as previously described.[9] After excised protein spots from 2D gels were digested with trypsin, the cleaved peptides were extracted from the gels using 50% (v/v) acetonitrile/5% (v/v) trifluoroacetic acid. The molecular mass of each peptide was measured using a matrix-assisted laser desorption ionization (MALDI)–time-of-flight (TOF)/TOF mass spectrometer (Shimadzu AXIMA-TOF2; Shimadzu, Japan). The obtained data were analyzed using the MASCOT program (Matrix Science, London, UK) to identify protein candidates for each spot. Database searches were carried out against the